REMARKS

Telephone Interview:

Applicants would like to express their appreciation to Examiner Kruse and Examiner Nelson for the courtesy extended to Angela Dallas during the telephone interview of September 11, 2002, during which the issues under 35 U.S.C. § 112, first paragraph were discussed.

Claim Amendments:

The claims have been amended to more particularly describe the invention. Support for the amendment to Claim 1 (and similarly amended claims) is found in Example 1, page 41, lines 13-15; page 47, lines 16-18; and page 52, lines 7-8. Support for the amendment to Claim 2 (and similarly amended claims) is found in the specification on page 8, lines 8-14. Support for added Claim 68 is found in Example 1, page 45, lines 9-14 and page 51, lines 20-25. Claims 66 and 67 were added to address the issue with regard to multiple dependencies raised by the Examiner. All other claim amendments are clerical in nature.

Claim Objections:

The Examiner has objected to Claims 44, 47 and 48 as being in improper dependent form. Claims 7, 39, 44, 47 and 48 have been amended to correct this issue.

Rejection of Claims 47 and 48 Under 35 U.S.C. § 112, Second Paragraph:

The Examiner has rejected Claims 47 and 48 under 35 U.S.C. § 112, second paragraph.

With regard to Claim 47, the Examiner contends that "a DNA construct" should read "the DNA construct". Claim 47 has been amended to adopt the Examiner's suggestion.

With regard to Claim 48, the Examiner contends that "which may comprise" is indefinite and also that "a DNA construct" should read "the DNA construct". Claim 48 has been amended to address the issue of indefiniteness and to adopt the Examiner's suggestion.

In view of the foregoing amendments and remarks, the Examiner is respectfully requested to withdraw the rejection of Claims 47 and 48 under 35 U.S.C. § 112, second paragraph.

Objection to the Specification and Rejection of Claims 1, 2, 4, 5, 7, 21, 24, 26, 39, 44, 47, 38, 50-52, 54-56 and 58-65 Under 35 U.S.C. § 112, First Paragraph:

The Examiner has objected to the specification and rejected Claims 1, 2, 4, 5, 7, 21, 24, 26, 39, 44, 47, 38, 50-52, 54-56 and 58-65 under 35 U.S.C. § 112, first paragraph, contending that these claims were not described in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s) at the time of the invention where in possession of the claimed invention. Specifically, the Examiner contends that a description of a method for obtaining DNA from a particular organism and the description of the encoded protein are insufficient to provide adequate written description of the actual DNA from the organism. In reference to the data provided in the Declaration of Dr. Weeks under 37 CFR 1.132 (filed July 27, 2001), the Examiner asserts that the conclusion that the DNA from three different strains encodes a dicamba-degrading oxygenase is based solely on the ability of the isolated DNA to bind to a probe based on SEQ ID NO:3 and not on empirical evidence that the strains actually contain a DNA encoding a dicamba-degrading oxygenase. Finally, the Examiner contends that for non-sequence based claims and for those describing an oxygenase having a specific homology to SEQ ID NO:4, a description of a molecule encoding an enzyme based solely on function does not constitute a proper description of that molecule.

Applicants again traverse the Examiner's rejection based on written description.

First, the specification provides a detailed description of the claimed sequences of the invention. With regard to the DNA molecules of Claim 1 and related molecules, Example 1 describes in detail the source of the enzyme and the structural, biochemical and functional properties of a dicamba-degrading oxygenase from a dicamba-degrading bacterium, as exemplified by *Pseudomonas*. With regard to sequence-based claims, the specification describes variants (mutants) of the exemplified sequence, including sequences that have one or more amino acid deletions (i.e., fragments) (see page 8, lines 8-14), and homologues of the exemplified sequences having 65% identity or 85% identity to the claimed sequence (see page 8, lines 16-22). The specification discloses at page 9, lines 12-17 how percent identity between two sequences can be determined. At page 10, line 20 to page 11, line 4; page 9, lines 18-28, the specification describes how molecular techniques can be used to identify and isolate additional clones from organisms other than the ones

from which the clones were originally isolated. At page 9, line 29, to page 10, line 9, or at page 11, lines 5-29, the specification shows how to produce mutant enzymes using recombinant DNA techniques and/or chemical synthesis techniques. At page 19, line 22 to page 20, line 12, as well as page 28, lines 6-29 and page 32, the specification shows how the biological activity of an oxygenase of the invention can be evaluated. Moreover, as discussed in the September 11 interview, the specification provides guidance to those of skill in the art regarding conserved structural features that would readily allow one of skill in the art to recognize what changes could be made to a given oxygenase which would not be expected to destroy the biological activity of the oxygenase. For example, page 40, line 27 to page 41, line 1 discloses that the dicamba-degrading oxygenase of the invention contains iron-sulfur cluster(s) or heme group(s), which are structural features known to be possessed by some oxygenases. Further, page 47, lines 12-15 discloses that comparison of the amino acid sequence of the dicamba-degrading oxygenase of the invention with that of the protein sequences in the public data base showed homology to other oxygenases, even though the overall sequence identity was relatively low (33.8% - see page 54, lines 3-6). Moreover, page 48, lines 1-18 discloses that the oxygenase of the invention possesses a subunit that is likely to exist as a dimer in its native state and that this $(\alpha)_n$-type subunit arrangement is similar to that found in other well characterized oxygenases, providing multiple citations to other known oxygenases. Finally, as discussed above, the specification provides a detailed discussion of the structural and biochemical features of the oxygenase from *Pseudomonas maltophilia* that provides additional information regarding features of an oxygenase that can be used to identify or evaluate a given oxygenase within the scope of the claims (Example 1).

In the September 11 interview, the Examiners suggested that evidence be provided with regard to the knowledge in the art at the time of the invention of conserved structural features of oxygenases. Such evidence is useful for establishing that one of skill in the art, given the sequence of the exemplified dicamba-degrading oxygenase, could readily identify which residues or domains of the protein should be preserved and which can be predictably modified without destroying the biological function of a given homologue. Enclosed herewith is a new Declaration of Dr. Donald Weeks under 37 CFR 1.132 (new Declaration), which provides multiple lines of evidence in support of Applicants' position that a major portion of the amino acids in the oxygenase_{DIC} of the present

invention (SEQ ID NO:4) can be changed to another amino acid (or to several different amino acids) without such changes being expected to substantially affect the enzymatic activity of the oxygenase.

Last, the Examiner's specific comments from the June 13 Office Action will be addressed. With regard the Examiner's statement that a description of a method for obtaining DNA from a particular organism and the description of the encoded protein are insufficient to provide adequate written description of the actual DNA from the organism, it is first noted that the non-sequence based claims have been amended to add additional structural, biochemical and functional properties to the claim that clearly define a dicamba-degrading oxygenase. The genus of dicamba-degrading oxygenases from dicamba-degrading bacteria having the recited characteristics is not expected to be highly variable because it is known in the art that bacteria have a high incidence of lateral gene similarity, because members of the genus (dicamba-degrading bacteria) are closely related functionally and because it is expected, referring now to the discussion below, that any dicamba-degrading oxygenases expressed by such bacteria will share conserved structural features in addition to the specified function. Therefore, the exemplification and description of the oxygenase that is recited in Claim 1 and related claims provides a clear written description that is sufficient to distinguish it from other proteins and convey to the skilled artisan that Applicants were in possession of such oxygenases at the time of the invention.

With regard to the Examiner's assertion that the DNA identified by Southern blot does not provide empirical evidence that the strains actually contain a DNA encoding a dicamba-degrading oxygenase, Applicants respectfully submit that such empirical evidence is not required, because the logical conclusion to be reached from the data is that other highly related dicamba-degrading oxygenases have been detected in the test bacteria. One of skill in the art would expect that these DNAs will encode oxygenases that degrade dicamba in view of the data provided. In contrast, the Examiner's conclusion is not supported by the most probable and logical interpretation of the data.

The rebuttal of the Examiner's conclusion is as follows. Applicants have demonstrated the existence of a dicamba-degrading oxygenase from a dicamba-degrading bacteria (i.e., SEQ ID NOs:3/4). Of sequences known prior to the invention, the sequence having the closest homology to the amino acid sequence of the invention is a different oxygenase that is only 33.8% identical to SEQ ID NO:4 (also referred to herein as oxygenase$_{DIC}$) . In the Southern blot described in the

Declaration of Dr. Weeks filed July 27, 2001 (July 27 Declaration), it was shown that a probe spanning 900 out of the 1020 bp of the oxygenase$_{DIC}$ DNA hybridized to DNA from each of three different dicamba-degrading bacterial strains, including from a different genus than *Pseudomonas*. Given the hybridization conditions and the size of the probe, it was estimated that the DNA that was hybridized in these strains had greater than 90% identity to the probe DNA (July 27 Declaration).

To maintain the Examiner's position, it must therefore be concluded that the *nearly full-length probe* from DNA encoding a dicamba-degrading oxygenase from a dicamba-degrading bacterium (oxygenase$_{DIC}$) would reasonably be expected to identify a DNA sequence in three different strains of dicamba-degrading bacteria that is at least 90% identical to the oxygenase$_{DIC}$ gene, but which is not actually a dicamba-degrading oxygenase gene, even though the next most closely related known oxygenase to oxygenase$_{DIC}$ is only 33.8% identical, and even though these bacteria are *expected to contain* a dicamba-degrading oxygenase (i.e., they are dicamba-degrading bacteria). The Examiner's conclusion further implies that the test DNA that hybridizes to the probe (but that allegedly does not encode a dicamba-degrading oxygenase) is more similar to the oxygenase$_{DIC}$ DNA of the invention than the DNA that actually *does* encode a dicamba-degrading oxygenase in the test bacteria. Finally, this conclusion also implies that the DNA that actually does encode a dicamba-degrading oxygenase in the test bacteria is so dissimilar to the oxygenase$_{DIC}$ DNA of the invention, that it does not hybridize to the probe. It is submitted that this highly improbable conclusion is not statistically or scientifically sound in view of the data.

Finally, with regard to the Examiner's contention that a description of a molecule encoding an enzyme based solely on function does not constitute a proper description of that molecule, with regard to non-sequence based claims, Applicants submit that the description of the DNA sequence encoding an oxygenase from a dicamba-degrading bacterium is not merely a description based on function because the source of the protein is identified and because the specification clearly describes the structural, biochemical, and physical properties of such an oxygenase. To expedite prosecution, non-sequence based claims have been amended to add such biochemical/structural features to the claim. With regard to those claims that recite a molecule with a given percent identity to another molecule, Applicants submit that the Examiner is incorrect in stating that the molecule is described solely based on function. To the contrary, a description of a sequence that is 65% identical, for

example, to a specific sequence is a structural definition which is clearly known to those of skill in the art. The combination of this structural definition with the functional limitation is sufficient to describe the claimed molecule.

In view of the foregoing remarks, the Examiner is respectfully requested to withdraw the rejection of Claims 1, 2, 4, 5, 7, 21, 24, 26, 39, 44, 47, 38, 50-52, 54-56 and 58-65 under 35 U.S.C. § 112, first paragraph.

Objection to the Specification and Rejection of Claims 1, 2, 4, 5, 7, 21, 24, 36, 39, 44, 47, 48, 50-52, 54, 55 and 58-65 Under 35 U.S.C. § 112, First Paragraph:

The Examiner has objected to the specification and rejected Claims 1, 2, 4, 5, 7, 21, 24, 36, 39, 44, 47, 48, 50-52, 54, 55 and 58-65 under 35 U.S.C. § 112, first paragraph, on the basis of enablement. The Examiner states that the specification is enabling for SEQ ID NO:3, for molecules encoding SEQ ID NO:4 and for methods of making and using such DNA molecules and plants comprising such molecules. However, the Examiner contends that the specification does not enable any DNA encoding a dicamba-degrading oxygenase as recited in the claims. The Examiner again refers to the discussion regarding the identification of DNA in other bacterial species using the probe based on SEQ ID NO:3 and infers that there is no empirical evidence that the strains actually contain a DNA encoding a dicamba-degrading oxygenase. With regard to the data for the transformed plant, the Examiner asserts that one of skill in the art can not reasonably predict that a plant transformed with any DNA sequence encoding a dicamba-degrading oxygenase would in fact be dicamba-resistant. The Examiner uses an analogy to a tobacco transformed with a soybean P450 monoxygenase, wherein the monoxygenase enhanced the plants' ability to metabolize several phenylurea herbicides, but did not result in the plant being resistant to all phenylurea herbicides.

With regard to the Examiner's analogy to a P450 monoxygenase, it is submitted that this is not an analogous scenario to the present claims and thus is not relevant. The scenario described by the Examiner refers to the question of whether a single monoxygenase is capable of catalyzing the degradation of several different substrates within a class (i.e., different phenylurea herbicides), thus speaking to the issue of whether the single enzyme can act on multiple different substrates. In contrast, the issue in the present invention is whether a single enzyme (i.e., a dicamba-degrading

oxygenase) can act on a single substrate, dicamba, which is one substrate within the class of benzoic acid herbicides. Applicants have demonstrated that an oxygenase that degrades dicamba confers dicamba resistance on a host transformed with the oxygenase. Therefore, there is no need to screen through all dicamba-degrading oxygenases to see whether they confer resistance or not. It is clearly predictable that if the oxygenase catalyzes the degradation of dicamba, then dicamba-resistance is predicted and reasonably expected of the transformed host. The Examiner has not provided a rationale as to why an oxygenase that degrades dicamba would not be expected to confer dicamba tolerance to a host organism.

In view of the foregoing remarks, the Examiner is respectfully requested to withdraw the rejection of Claims 1, 2, 4, 5, 7, 21, 24, 36, 39, 44, 47, 48, 50-52, 54, 55 and 58-65 under 35 U.S.C. § 112, first paragraph.

Applicants have attempted to respond to all of the Examiner's concerns and submit that the claims are in a condition for allowance. If the Examiner has any questions or concerns regarding Applicants' position, contact of the below-named agent is encouraged.

Respectfully submitted,

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Date: December 13, 2002

Claims 1, 2, 4, 5, 7, 21, 36, 39, 44, 47, 48, 59 and 63 have been amended as shown below.

Claims 3, 6, 22-24, 37, 38, 50-58, 60-62, 64 and 65 have not been changed.

Claims 66-68 have been added.

1. (Three Times Amended) An isolated DNA molecule comprising a DNA sequence encoding a dicamba-degrading oxygenase from a dicamba-degrading bacterium, wherein said dicamba-degrading oxygenase has a subunit molecular mass of about 40kD, comprises an iron-sulfur cluster, and catalyzes the oxidation of dicamba to 3,6-dichlorsalicylic acid (DCSA).

2. (Three Times Amended) An isolated DNA molecule comprising a DNA sequence encoding a dicamba-degrading oxygenase, wherein said dicamba-degrading oxygenase is selected from the group consisting of:

a. a dicamba-degrading oxygenase having the amino acid sequence of SEQ ID NO:4; [and]

b. a fragment of SEQ ID NO:4 that has dicamba-degrading oxygenase activity; and

c. a dicamba-degrading oxygenase having an amino acid sequence which is at least about 65% identical to the amino acid sequence of SEQ ID NO:4 and which has dicamba-degrading oxygenase activity.

4. (Three Times Amended) A DNA construct comprising a DNA sequence encoding a dicamba-degrading oxygenase from a bacterium that degrades dicamba operatively linked to expression control sequences, wherein said dicamba-degrading oxygenase has a subunit molecular mass of about 40kD, comprises an iron-sulfur cluster, and catalyzes the oxidation of dicamba to 3,6-dichlorsalicylic acid (DCSA).

5. (Three Times Amended) A DNA construct comprising a DNA sequence encoding a dicamba-degrading oxygenase operatively linked to expression control sequences, wherein said dicamba-degrading oxygenase is selected from the group consisting of:

a. a dicamba-degrading oxygenase having the amino acid sequence of SEQ ID NO:4; [and]

b. a fragment of SEQ ID NO:4 that has dicamba-degrading oxygenase activity; and

c. a dicamba-degrading oxygenase having an amino acid sequence which is at least about 65% identical to the amino acid sequence of SEQ ID NO:4 and which has dicamba-degrading oxygenase activity.

7. (Twice Amended) The DNA construct of Claim [4 or] 5 which is a vector.

21. (Three Times Amended) A transgenic host cell comprising DNA encoding a dicamba-degrading oxygenase, said DNA being operatively linked to expression control sequences;

wherein said dicamba-degrading oxygenase is selected from the group consisting of:

a. a dicamba-degrading oxygenase having the amino acid sequence of SEQ ID NO:4; [and]

b. a fragment of SEQ ID NO:4 that has dicamba-degrading oxygenase activity; and

c. a dicamba-degrading oxygenase having an amino acid sequence which is at least about 65% identical to the amino acid sequence of SEQ ID NO:4 and which has dicamba-degrading oxygenase activity.

36. (Three Times Amended) A transgenic plant or part of a plant comprising one or more cells comprising DNA encoding a dicamba-degrading oxygenase, said DNA being operatively linked to expression control sequences;

wherein said dicamba-degrading oxygenase is selected from the group consisting of:

a. a dicamba-degrading oxygenase having the amino acid sequence of SEQ ID NO:4; [and]

b. a fragment of SEQ ID NO:4 that has dicamba-degrading oxygenase activity; and

c. a dicamba-degrading oxygenase having an amino acid sequence which is at least about 65% identical to the amino acid sequence of SEQ ID NO:4 and which has dicamba-degrading oxygenase activity.

39. (Twice Amended) The transgenic plant or plant part of Claim 36 [or 62] wherein the plant is a broadleaf plant which is tolerant to dicamba as a result of the expression of the dicamba-degrading oxygenase and the plant part is a part of a broadleaf plant which is tolerant to dicamba as a result of the expression of the dicamba-degrading oxygenase.

44. (Three Times Amended) A method of controlling weeds in a field containing a transgenic plant according to any one of Claims 36-39, [or]61-62 or 64-65 comprising applying an amount of dicamba to the field effective to control the weeds in the field.

47. (Three Times Amended) A method of selecting transformed plant cells comprising:

providing a population of plant cells;

transforming at least some of the plant cells in the population of plant cells with [a] the DNA construct according to any one of Claims 4-7, [or 53]54-56 or 66; and

selecting the transformed plant cells by culturing the resulting population of plant cells in a culture medium containing dicamba at a concentration selected so that transformed plant cells proliferate and untransformed plant cells do not proliferate.

48. (Three Times Amended) A method of selecting transformed plants comprising:

providing a population of plants which [may comprise] comprises one or more plants comprising [a] the DNA construct according to any one of Claims 4-7, [or 53]54-56 or 66; and

selecting transformed plants by applying an amount of dicamba to the population of plants selected so that transformed plants grow, and growth of untransformed plants is inhibited.

59. (Once Amended) A transgenic host cell comprising DNA encoding a dicamba-degrading oxygenase from a bacterium that degrades dicamba, said DNA being operatively

linked to expression control sequences, wherein said dicamba-degrading oxygenase has a subunit molecular mass of about 40kD, comprises an iron-sulfur cluster, and catalyzes the oxidation of dicamba to 3,6-dichlorsalicylic acid (DCSA).

63. (Once Amended) A transgenic plant or part of a plant comprising one or more cells comprising DNA encoding a dicamba-degrading oxygenase from a bacterium that degrades dicamba, said DNA being operatively linked to expression control sequences, wherein said dicamba-degrading oxygenase has a subunit molecular mass of about 40kD, comprises an iron-sulfur cluster, and catalyzes the oxidation of dicamba to 3,6-dichlorsalicylic acid (DCSA).

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application of:	Group Art Unit: 1638
WEEKS et al.	Examiner: D. Kruse
Serial No.: 09/055,145	DECLARATION OF DR. DONALD P. WEEKS (Under 37 CFR 1.132)
Filed: April 3, 1998	
Atty. File No.: 3553-18	
For: "METHODS AND MATERIALS FOR MAKING AND USING TRANSGENIC DICAMBA-DEGRADING ORGANISMS"	

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Dr. Donald P. Weeks, declare that:

1. I am the same Donald P. Weeks who is named as an inventor on the above-refereuced patent application.

2. The following discussion and attached references and alignments are presented in support of Applicants' arguments against the rejection of Claims 1, 2, 4, 5, 7, 21, 24, 26, 39, 44, 47, 38, 50-52, 54-56 and 58-65 under 35 U.S.C. § 112, first paragraph. Specifically, the following discussion provides additional evidence that a major portion of the amino acids in the oxygenase$_{DIC}$ of the present invention (SEQ ID NO:4) can be changed to another amino acid or, in many cases, to several different amino acids, without such changes being expected to substantially affect the enzymatic activity of the oxygenase.

The following attachments are referenced in the discussion below:

1) Voet and Voet, 2nd edition, 1995, John Wiley & Sons, Inc., Chapter 6, pages 152-130;

2) Gibson and Parales, 2000, *Curr. Opin. Biotechnol.* 11:236-243;

3) Jiang et al., 1996, *J. Bacteriol.* 178:3133-3139;

4) van der Geize et al., 2002, *Mol. Microbiol.* 45:1007-1018;

5) Figure A, Weeks et al., 2002, from a manuscript in preparation

(a) Discussion and Evidence Regarding Primary Sequence Variation in the Art

First, it is a widely known fact among biochemists and molecular biologists that a particular protein with a specific enzymatic activity can possess several different primary amino acid sequences and still maintain the enzymatic activity. It is often seen that only a few amino acids are "invariant" within a particular family of proteins with the same or similar enzymatic activities. These invariant amino acids often are part of a consensus sequence at the active site of the enzyme or at a particular structural domain(s) that is essential for enzymatic activity. At most other sites within the protein, it is typically the case that another amino acid can be substituted without having a significant effect on the activity of the enzyme. This is especially true when a "conservative substitution" is made. That is, when an amino acid with similar biochemical properties (e.g., size, charge, hydrophilicity/hydrophobicity, side chains, etc.) is substituted, there is a good probability that there will be no significant negative effect on the activity of the resultant enzyme relative to the "wild-type" or "parent" enzyme.

Knowledge of the phenomena described above is widespread. Attached to this declaration are pages copied from a standard Biochemistry textbook (Voet and Voet, 2nd edition, 1995) that describe the wide spread existence of proteins with the exact or similar function, but with significant variability in primary amino acid sequence. The specific example highlighted in this textbook is cytochrome c, a protein that is essential to energy production in all eukaryotic organisms from yeast to mammals. Examination of this protein indicates that its amino acid sequence has been moderately conserved over evolutionary time. This is relevant to the present invention because, based on analyses of other oxygenases with similar characteristics to oxygenase$_{DIC}$ (SEQ ID NO:4) (e.g., Gibson and Parales, 2000), one of skill in the art will conclude that it is likely that oxygenase$_{DIC}$ will be moderately conserved

within the bacterial community that harbors its activity (i.e., it may possess the kind of variability in amino acid sequence as seen for cytochrome c).

In the case of cytochrome c, examination of Table 6-4 on page 126 of the Voet and Voet textbook reveals that amino acid substitutions can be observed in nature in nearly one-half (**50%**) of the positions within the amino acid sequence while maintaining the enzymatic activity of the enzyme. Moreover, it is highly likely that additional amino acid substitutions could be made without an effect on the function of cytochrome c. This is because the cytochrome c molecules from only a small portion of the eukaryotic kingdom have been analyzed to date and because, as noted above, biochemists know that substitution of amino acids with similar properties are often well tolerated in regard to retention of enzymatic activities. Note that even in the limited number of cytochrome c sequences depicted in Table 6-4, there are individual sites at which six or seven different amino acids can substitute perfectly well.

(b) Evidence Regarding the Structure-to-Function Relationship of the Oxygenase$_{DIC}$ (SEQ ID NO:4)

Turning now to the oxygenase$_{DIC}$ (SEQ ID NO:4) of the present invention, I and my coinventors have strong evidence that oxygenase$_{DIC}$ is a member of the Rieske, non-heme iron oxygenases according to criteria set forth by Dr. David Gibson, one of the leading experts in the field of bacterial degradation of natural and synthetic aromatic chemicals (Gibson and Parales, 2000). First, it is noted that in the specification at page 48, lines 1-18, it is described that the subunit structure of the oxygenase$_{DIC}$ is similar to several of the other known oxygenases, including oxygenases that fall within this class (e.g., 3-chlorobenzoate 3,4-dioxygenase). Examination of the biochemical and physical properties of oxygenase$_{DIC}$, as well as its primary amino acid sequence, reveals that it contains two essential elements, a Rieske iron-sulfur cluster (see the specification at page 40, line 27 to page 41, line 1, wherein this iron-sulfur cluster is first identified) and a mononuclear iron binding site (page 52, lines 15-18, for example, discloses the requirement for iron (Fe^{2+}) for

activity). As discussed in the attached publication by Jiang et al., at the time of the invention, information regarding the structure-to-function relationship of oxygenases was available to those of skill in the art. Indeed, the specification (pages 48 and 54) discloses similarities between the oxygenase$_{DIC}$ and such known oxygenases. Therefore, by alignment of the oxygenase$_{DIC}$ sequence of the present invention with other known oxygenases of similar subunit structure, one of skill in the art can readily predict which amino acid positions can be modified while expecting to maintain enzymatic activity.

The amino acid sequence for the oxygenase$_{DIC}$ (SEQ ID NO:4) exhibits two highly conserved consensus sequences that are separately involved in the binding of the Rieske cluster and the "free" iron molecule (see attached Figure A). Alignment of these sequences with sequences from oxygenases from other bacteria illustrate that the consensus sequences of oxygenase$_{DIC}$ are quite similar to those in several different genera of bacteria. It is noted that two of the related sequences provided in the attached alignment were discussed in the specification with regard to the structure of the oxygenase of the invention (i.e., 3-chlorobenzoate 3,4-dioxygenase (CbaA) on page 48; and the closest sequence homologue at 33.8% identity, the oxygenase component of vanillate demethylase (VanA), see page 54).

It is important to note that there can be some degree of variability even within the consensus sequence. For example, within the binding site for mononuclear iron, the first "invariant" amino acid in the consensus sequence is usually glutamic acid (see Figure 3 in the attached publication by Jiang et al., page 3135). However, in oxygenase$_{DIC}$, the first amino acid is aspartic acid, a characteristic it shares with the oxygenase component from vanillate demethylase (VanA; see Fig. 3 of Jiang et al.) and a few other recently characterized oxygenases (see Figure 4 in the attached publication by van der Geize et al., page 1011). Furthermore, even within the domains that comprise the consensus sequence, except for a few invariant amino acids at specific locations, most of the positions can be occupied by a variety of different amino acids. Indeed, it is readily apparent from the alignment shown in Fig.

A and the alignments shown in the publications that greater than 50% of the sites in the sequences shown can be occupied by a variety of amino acids while expecting that oxygenase activity is maintained.

(c) Further Evidence of Variability of Structure Among Naturally Occurring Enzymes Having the Same Function

The present inventors have now discovered two different amino acid sequences for reductase$_{DIC}$, the purified protein which was described in the present application. These reductase genes are from the same bacterium and encode proteins of the same function, and yet they differ in at least five different places in their amino acid sequence (primarily at the beginning (i.e., N-terminus) and end (C-terminus) of the enzyme (sequences are attached hereto). This data provides a direct indication that it is likely that oxygenases$_{DIC}$ from even very closely related bacteria that contain differences in amino acid sequences will maintain the functionality of the enzyme. The more distantly related the bacterial species in time, distance and environment, the more likely they will contain an oxygenase$_{DIC}$ that will be different in amino acid sequence – and yet retain exactly the same enzymatic activity.

(d) Summary

From the above discussion, it is clear to those of skill in the art that a major portion of the amino acids in oxygenase$_{DIC}$ can be predictably changed to another amino acid (or perhaps to several different amino acids) without affecting the enzymatic activity of the enzyme in a significant fashion. When this is coupled with knowledge that several efficient methods for site-directed mutagenesis of gene sequences are available and are practiced routinely in thousands of laboratories around the world, there is an inescapable conclusion that one skilled in the art can modify the amino acid sequence of the oxygenase$_{DIC}$ enzyme, including easily up to 35% (i.e., a homologue having 65% identity), without inhibiting the ability of the enzyme to convert the active herbicide, dicamba, to the herbicidally-inactive compound, 3,6-dichlorsalicylaic acid (DCSA).

3. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: December 12, 2002 By: _____

Dr. Donald P. Weeks